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# Exclusion of Linkage Between Bipolar Affective Disorder and Chromosome 16 in 12 Australian Pedigrees

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**Several recent reports of possible susceptibility loci for bipolar affective disorder (BAD) have identified sites on a number of chromosomes. Specifically, two Danish studies have suggested the presence of a susceptibility locus for BAD on chromosome 16p13. As the first step of a whole genome scan, we screened 12 Australian families with markers at 16p13 and also a number of markers spanning the entirety of chromosome 16. Linkage analysis was undertaken using both the parametric lod score method (two- and multipoint) with different models and diagnostic thresholds, and the nonparametric affected pedigree member (APM) method. Results of lod score analysis convincingly excluded the 16p13 region from linkage to BAD in these families, while APM provided no support for linkage. Furthermore, using the broad definition of BAD, with individuals affected by bipolar I and II and recurrent unipolar disorders included, the entire chromosome was excluded from linkage to BAD with autosomal-dominant transmission at a maximum age-specific penetrance of 60%, and with autosomal-dominant and recessive modes of transmission at a maximum age-specific penetrance level of 90%. Diagnostic thresholds which did not include unipolar affected individuals were somewhat less informative. However, a majority (between 63–96%, depending upon the model) of the chromosome was clearly excluded using narrow diagnostic thresholds. Moreover, no positive lod scores were obtained at θ = 0.00 for any tested model or diagnostic threshold. Our results indicate that no linkage exists between BAD and chromosome 16 markers in this group of Australian families. Am. J. Med. Genet. 74:304–310, 1997.** © 1997 Wiley-Liss, Inc.

**KEY WORDS: manic depressive illness; depression; genome scan; genetic linkage**

## INTRODUCTION

Bipolar affective disorder (BAD) is a condition characterized by severe mood disturbances and variable age of onset. It affects between 1–2% of the population, and may occur in both a familial and a sporadic manner. Although twin and family studies indicate that BAD has a strong genetic basis, the number of genes and mode of transmission of the disorder are poorly understood.

Recent research has generated conflicting reports regarding the number of susceptibility loci for BAD. Evidence suggesting that a single major locus is responsible for BAD has been reported by Spence et al. [1995]. In contrast, investigations by Craddock et al. [1995] suggest that a single major locus, or a multilocus heterogeneity model, is not consistent with BAD population and family data. Their data is suggestive of a multiplicative model involving three or more loci [Craddock et al., 1995]. Interestingly, earlier work by Cox et al. [1989] implied that a single major locus may cause susceptibility to BAD when bipolar I (BPI) and schizoaffective patients are regarded as affected. In contrast, more than one gene may lead to susceptibility when hypomania (bipolar II; BPII) or recurrent major depression (unipolar disorder, UP) patients are included in the affection status.

Many groups are currently investigating the genetic basis of BAD, using both parametric and nonparamet-

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ric methods of analysis. Considerable interest has focused on chromosome 18, as first reported by Berrettini et al. [1994], and subsequently by Stine et al. [1995], Freimer et al. [1996], Coon et al. [1996], and De bruyn et al. [1996]. Other studies suggest the presence of a susceptibility locus for BAD on chromosome 21 [Straub et al., 1994; Gurling et al., 1995; Detera-Wadleigh et al., 1996]. Recent reports have added new dimensions to the search for BAD susceptibility loci, with putative loci reported on chromosomes 4, 6, 13, and 15 [Blackwood et al., 1996; Ginns et al., 1996].

Two intriguing reports also suggest a possible susceptibility locus on chromosome 16. Eiberg et al. [1993] reported a suggestion of linkage between BAD and the phosphoglycolate phosphatase (PGP) locus at 16p13.3. Assuming an autosomal-dominant mode of transmission, a maximum lod score of $z = 2.20$ was identified at a recombination fraction of $\theta = 0.00$ in a large Danish family. Further studies by this group supported the hypothesis using microsatellite markers [Ewald et al., 1995]. They obtained a maximum lod score of $z = 2.52$ in two Danish families for D16S510, a marker which lies approximately 6 cM proximal to the PGP locus. In contrast to the original report by Eiberg et al. [1993], which described a maximal score using autosomal-dominant transmission, the maximum lod score reported by Ewald et al. [1995] was achieved assuming an autosomal-recessive mode of transmission. Interestingly, lod scores for the D16S291 marker (located in the same region as the PGP locus) were found to be negative [Ewald et al., 1995].

In this report we present a linkage analysis of the whole of chromosome 16, the first chromosome to be completed in our ongoing full genome screen, with particular emphasis on the putative susceptibility region of 16p13 in 12 moderate-to-large-sized Australian pedigrees.

## MATERIALS AND METHODS

Twelve pedigrees, which were recruited between 1987–1992 [Mitchell et al., 1991, 1992], were selected on the basis of having a large number of relatives, a single disease source, illness in at least two generations, a large number of affected members (at least 3 affected, with a minimum of 2 bipolar I patients), and large sibships. Details of pedigrees were recorded using the method described by Thompson et al. [1979]. The families were assessed using the Family History Research Diagnostic Criteria (FH-RDC) method of Andreasen et al. [1977], with interviews performed with both the proband and another informative family member.

All available first- and second-degree relatives of the proband were interviewed using standardized instruments to derive lifetime RDC diagnoses. Strict NIMH criteria for depression, which require at least 1 month's duration of symptoms and functional impairment or incapacity, were employed. Family 01 was assessed using the Schedule of Affective Disorders and Schizophrenia-Lifetime Version (SADS-L). The remaining 11 families were assessed with the Composite International Diagnostic Interview (CIDI) [Robins et al., 1988], allowing the application of multiple diagnostic systems (RDC, DSM-III, DSM-IIIR, and ICD-10) to linkage analysis.

In conjunction with the structured interviews, all marrying-in individuals were routinely questioned about any family history of psychiatric disorder to ensure unilineal descent of illness in the pedigrees. Families were considered bilineal (and therefore excluded) if there was either a history of bipolar disorder in the "marrying-in" spouse, or a first- or second-degree relative of that spouse; or a history of recurrent major depression in the "marrying-in" spouse.

Hospital and other medical records were obtained, and final diagnoses were formed based on FH-RDC and structured interview-derived RDC diagnoses and medical records using the Yale-NIMH "Best Estimate" Diagnosis Consensus guidelines. The interviews were conducted by trained psychologists and psychiatric nurses. Consensus "best estimate" diagnoses were made after independent evaluation of all available interviews and records by psychiatrists P.B.M. and B. Waters.

DNA was extracted from peripheral blood lymphocytes and used for genotype analysis of 12 markers located on chromosome 16 (Fig. 1). The first chromosome 16 marker reported to be linked to BAD, i.e., PGP [Eiberg et al., 1993], is located close to D16S291. The D16S510 marker described by Ewald et al. [1995] is localized to the same region as D16S423. A total of between 166–199 individuals from the 12 families were genotyped for each marker, including 59 affected members (35 bipolar I (BPI), 5 bipolar II (BPII), and 19 recurrent major depression (UP)). Polymerase chain reaction (PCR) amplification was undertaken as previously described by Le et al. [1994]. Primer sequences were obtained from the Genome Database (GDB). Polymorphism information content (PIC) values were calculated for each marker, and ranged between 0.61–0.86 (Fig. 1). Samples were electrophoresed on 6% polyacrylamide gels and independently genotyped by two people with no prior knowledge of affection status.

Two-point linkage analyses using the MLINK program of the LINKAGE package, version 5.22 [Lathrop et al., 1984], were undertaken by calculating lod scores at each of the 12 markers examined. Two models incorporating age-dependent liability classes and different age-specific penetrance levels were used for data analysis [Le et al., 1994]. Maximum age-specific penetrance levels were taken as 60% for model 1 (model 1 of Le et al. [1994]) and 90% for model 2 (model 4 of Le et al. [1994]). The 90% maximum age-specific penetrance level used in model 2 assumes a relatively large genetic contribution to BAD. The 60% level used in model 1 allows for a greater environmental influence than model 2. Each of these models was analyzed assuming both autosomal-dominant and autosomal-recessive modes of inheritance. The diagnostic thresholds examined with each model were 1) BPI, BPII, and UP, 2) BPI and BPII, and 3) BPI only. The frequency of the disease allele was defined as 0.035 for the dominant model and 0.2 for the recessive model, and the sporadic rate was set at 0.005 for both models. The maximum power of the 12 families to detect linkage

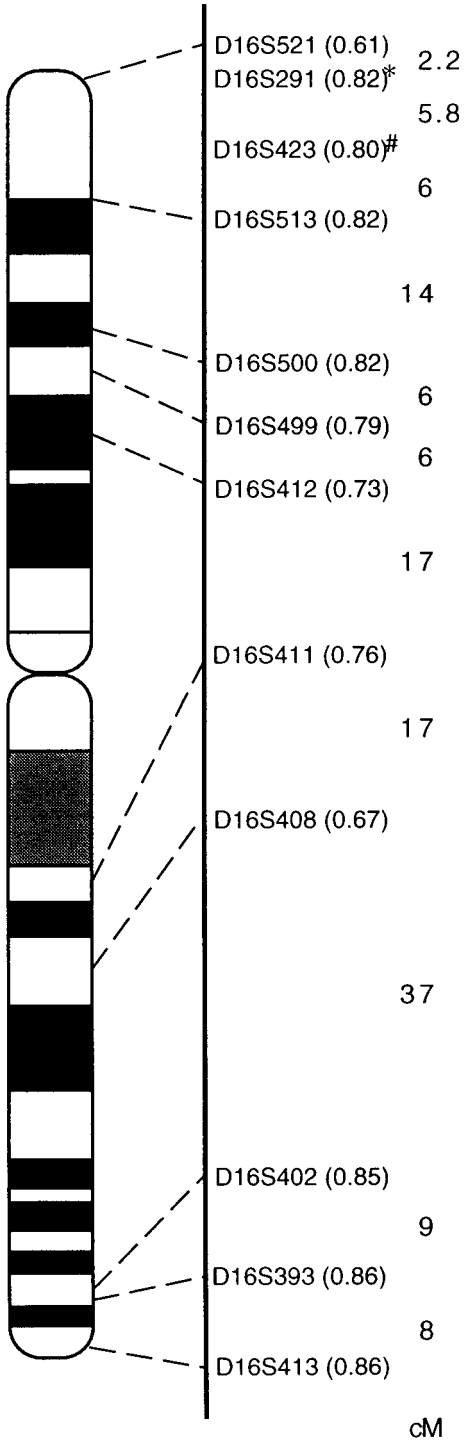


Fig. 1. Cytogenetic and linkage maps of chromosome 16 from Gyapay et al. [1994] and the Genome Database. Relative positions of each of the 12 markers used for this investigation, and the approximate distance (in cM) between markers, are indicated. D16S291 is located in close proximity to the PGP locus (asterisk). D16S423 is located in the same region as D16S510 (pound sign). Number in brackets alongside each marker is the PIC.

was determined by SLINK [Ott, 1989; Weeks et al., 1990]. Under the assumption of homogeneity, these pedigrees give a maximum lod score of 29.9, and an average maximum score of 12.8. An average maximum lod score of 7.15, and maximum lod score of 29.7, result from analysis using the assumption of 50% heterogeneity for the pedigrees.

Multipoint analysis (using the LINKMAP program of the LINKAGE package, version 5.22) was undertaken in the regions spanning markers for which linkage was not excluded by two-point analyses. The region between each adjacent pair of markers was examined at five evenly spaced points. Data analysis was performed using the same models as for two-point analyses.

The affected pedigree member (APM) method was also utilized for data analysis [Weeks and Lange, 1988]. This method tests for the identity-by-descent (IBD) of the disease alleles and does not require assumed knowledge of the mode of transmission. However, APM analysis is highly dependent on marker allele frequencies. The three test statistics, $f(p) = 1$, $f(p) = 1/\sqrt{p}$, and $f(p) = 1/p$, as described by Weeks and Lange [1988], were used for APM analysis. As an index of the Australian population prevalence, marker allele frequencies used in both APM and linkage analysis were determined from the "marrying-in" spouses and one other individual randomly chosen from each pedigree. Sib-pair analysis was not undertaken, as there was an insufficient number of affected sib-pairs in the sample to provide informative results.

## RESULTS

### Lod Scores

Two-point lod scores were calculated using models 1 (60% penetrance) and 2 (90% penetrance) for each marker and with the three diagnostic classes. Lod scores for recombination fractions of $\theta = 0.00$ and $\theta = 0.05$ are presented in Table I. Examination of these results clearly shows no evidence for linkage of any of the markers to BAD. In fact, most of the markers could be clearly excluded from linkage, as their lod scores at $\theta = 0.00$ were $<-2.0$. However, all of the markers for which linkage was not excluded had two-point lod scores of $<0$.

A graphical representation of the two-point linkage analyses for models 1 and 2, showing both dominant and recessive modes of transmission, illustrates the overall chromosomal picture (Fig. 2). Data generated for the broad (BPI, BPII, and UP) diagnostic threshold showed that between 87–100% of the chromosome was excluded from linkage to BAD. The exact level of exclusion was model-dependent. The percentage of chromosomal exclusion was less when data were analyzed with the narrower diagnostic thresholds (BPI and BPII (data not shown), and BPI only). However, in each instance, a majority of the chromosome was excluded.

The region spanning D16S402–D16S408–D16S411–D16S412 was not completely excluded from linkage to BAD with all of the two-point analyses. Hence, a multipoint analysis of this region was undertaken. Combined two- and multipoint results obtained using the broad diagnostic threshold excluded linkage of BAD to this entire region for all models except model 1 (60% penetrance) with a recessive mode of transmission. However, this model still only had a maximum lod score of –1.42 for D16S402–D16S408. As would be ex-

TABLE I. Lod Scores Obtained for Models 1 (60% Maximum Age-Specific Penetrance) and 2 (90% Maximum Age-Specific Penetrance) With Both Dominant and Recessive Modes of Inheritance at Recombination Fractions of $\theta = 0.00$ and $\theta = 0.05$ for the Three Diagnostic Thresholds*

| | Recombination fraction | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Model 1/Dom | | Model 2/Dom | | Model 1/Rec | | Model 2/Rec | |
| Diagnosis | 0.00 | 0.05 | 0.00 | 0.05 | 0.00 | 0.05 | 0.00 | 0.05 |
| BPI, BPII, and UP | | | | | | | | |
| D16S521 | −5.4 | −2.7 | −9.5 | −4.2 | −1.0 | 0.1 | −2.6 | −0.3 |
| D16S291[a] | −4.7 | −2.2 | −10.9 | −3.9 | −5.1 | −2.9 | −11.5 | −5.5 |
| D16S423[b] | −6.3 | −3.4 | −10.9 | −4.6 | −5.3 | −3.8 | −10.3 | −5.9 |
| D16S513 | −3.4 | −1.7 | −8.5 | −3.3 | −4.2 | −2.5 | −12.6 | −6.4 |
| D16S500 | −4.9 | −2.8 | −13.4 | −6.6 | −4.4 | −2.5 | −9.1 | −4.6 |
| D16S499 | −8.1 | −4.3 | −14.8 | −7.8 | −3.9 | −2.5 | −9.9 | −6.0 |
| D16S412 | −1.7 | −0.7 | −8.3 | −4.4 | −2.5 | −1.3 | −6.1 | −2.9 |
| D16S411 | −8.3 | −3.9 | −14.6 | −5.6 | −5.0 | −3.3 | −13.0 | −7.0 |
| D16S408 | −8.8 | −5.0 | −14.9 | −8.2 | −5.7 | −3.6 | −11.5 | −6.5 |
| D16S402 | −8.6 | −5.6 | −18.7 | −11.6 | −6.0 | −3.5 | −12.7 | −6.8 |
| D16S393 | −5.9 | −3.7 | −13.9 | −8.4 | −5.5 | −3.4 | −12.8 | −7.2 |
| D16S413 | −12.3 | −6.9 | −23.0 | −10.1 | −7.5 | −4.9 | −18.4 | −10.7 |
| BPI and BPII | | | | | | | | |
| D16S521 | −3.6 | −2.1 | −10.2 | −5.8 | −1.5 | −0.7 | −4.7 | −2.6 |
| D16S291[a] | −3.1 | −1.5 | −9.2 | −5.2 | −3.5 | −2.1 | −7.5 | −3.9 |
| D16S423[b] | −6.5 | −4.4 | −11.2 | −7.5 | −4.2 | −2.7 | −6.3 | −4.1 |
| D16S513 | −6.0 | −3.8 | −9.8 | −6.3 | −5.6 | −3.4 | −10.6 | −5.7 |
| D16S500 | −6.1 | −3.3 | −11.9 | −6.4 | −6.3 | −4.1 | −11.9 | −7.3 |
| D16S499 | −7.4 | −3.8 | −10.2 | −6.5 | −5.1 | −3.5 | −10.5 | −6.9 |
| D16S412 | −3.6 | −1.3 | −10.2 | −5.1 | −1.5 | −0.8 | −4.4 | −2.2 |
| D16S411 | −7.2 | −2.5 | −10.7 | −4.5 | −5.2 | −3.2 | −9.5 | −5.5 |
| D16S408 | −2.6 | −1.4 | −7.1 | −4.5 | −3.6 | −2.5 | −7.4 | −4.5 |
| D16S402 | −5.1 | −2.5 | −7.7 | −4.7 | −3.9 | −2.6 | −7.9 | −4.9 |
| D16S393 | −5.0 | −3.4 | −12.1 | −6.8 | −4.1 | −2.5 | −10.6 | −5.7 |
| D16S413 | −9.6 | −5.8 | −18.3 | −10.0 | −8.3 | −5.6 | −15.7 | −9.5 |
| BPI only | | | | | | | | |
| D16S521 | −4.0 | −2.3 | −8.3 | −4.6 | −0.7 | −0.3 | −3.4 | −2.0 |
| D16S291[a] | −3.1 | −1.6 | −8.7 | −4.9 | −3.0 | −1.9 | −5.9 | −3.4 |
| D16S423[b] | −6.7 | −4.3 | −9.9 | −6.6 | −4.3 | −2.8 | −6.5 | −4.1 |
| D16S513 | −6.4 | −3.8 | −9.8 | −5.1 | −5.7 | −3.6 | −11.1 | −6.4 |
| D16S500 | −4.2 | −2.2 | −9.8 | −5.2 | −5.8 | −4.0 | −10.3 | −6.8 |
| D16S499 | −5.7 | −3.1 | −8.1 | −6.0 | −5.4 | −3.7 | −10.4 | −6.9 |
| D16S412 | −1.4 | −0.7 | −6.5 | −3.7 | −1.0 | −0.5 | −2.7 | −1.5 |
| D16S411 | −4.0 | −0.5 | −6.4 | −1.6 | −4.8 | −2.8 | −8.9 | −5.1 |
| D16S408 | −2.5 | −1.4 | −6.5 | −4.0 | −2.1 | −1.4 | −4.9 | −2.4 |
| D16S402 | −5.5 | −2.9 | −7.0 | −4.8 | −3.0 | −2.2 | −7.2 | −4.8 |
| D16S393 | −6.1 | −4.2 | −11.1 | −5.9 | −4.3 | −2.8 | −10.7 | −5.9 |
| D16S413 | −9.7 | −6.2 | −15.8 | −8.8 | −8.0 | −5.5 | −13.8 | −8.7 |

*Dom, dominant; Rec, recessive.
[a]D16S291 is located in close proximity to the PGP locus.
[b]D16S423 is located in the same region as D16S510.

pected, scores obtained using the narrower diagnostic thresholds were generally less negative than those for the broad diagnostic threshold.

Of the two chromosome 16 markers previously reported to be linked to BAD, i.e., PGP and D16S510, linkage to D16S291 (localized to the same region as PGP) and to D16S423 (adjacent to D16S510) was excluded using all models and diagnostic thresholds. Linkage to BAD was also excluded for each model and diagnostic threshold across the entire region spanned by these two markers (Fig. 2).

### APM Analysis

All three of the test statistics described by Weeks and Lange [1988] were calculated for each diagnostic threshold. Scores obtained for the $f(p) = 1/\sqrt{p}$ weighting function were generally found to be intermediate to those of the $f(p) = 1$ and $f(p) = 1/p$ statistics (data available on request). The most significant score obtained was for D16S411, using the $f(p) = 1/p$ weighting function at each diagnostic threshold. A maximum value of 3.74 and a $P$ value of 0.00009 for the BPI-only threshold was obtained. However, this score was not significant for the $f(p) = 1/\sqrt{p}$ or $f(p) = 1$ weighting functions, suggesting that this result was due to the weighting of allele frequencies and not of general significance. No other APM statistics were significant, including those for the D16S291 and D16S423 markers, for which $P > 0.05$.

## DISCUSSION

Parametric (lod scores) and nonparametric (APM method) analyses of 12 markers on chromosome 16 showed no evidence of linkage to BAD in 12 Australian pedigrees. Furthermore, combined two- and multipoint

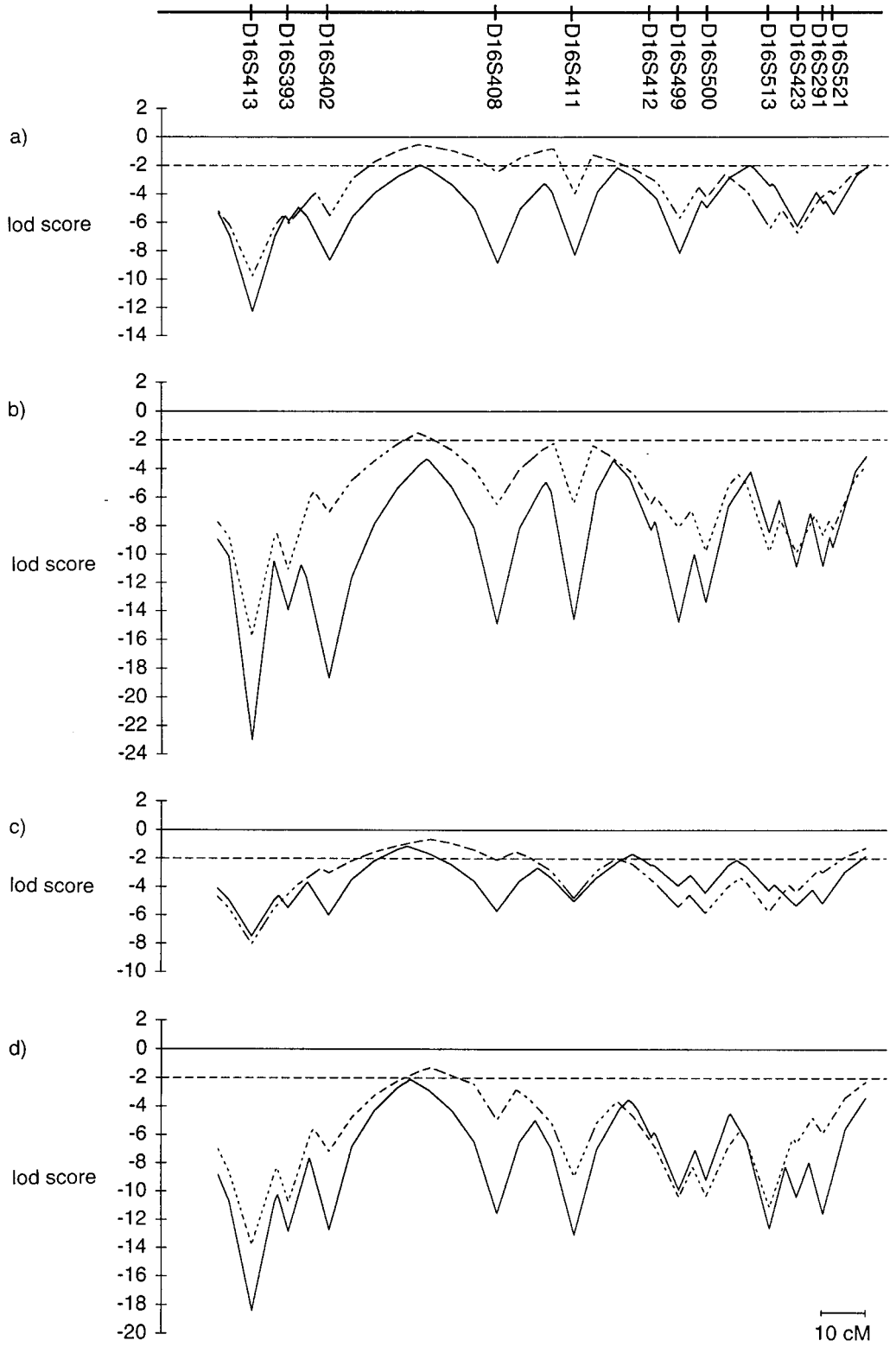


Fig. 2. Results of two-point linkage analysis for broad (BPI, BPII, and UP) and narrow (BPI only) analyses, using models 1 (maximum age-specific penetrance of 60%) and 2 (maximum age-specific penetrance of 90%) at each of the 12 markers examined. **a:** Model 1, dominant transmission. **b:** Model 2, dominant transmission. **c:** Model 1, recessive transmission. **d:** Model 2, recessive transmission. BPI, BPII, and UP combined data are indicated by solid lines, and BPI-only data by dashed lines.

linkage analyses using different diagnostic thresholds and inheritance models excluded a large proportion of chromosome 16 from linkage to BAD. In particular, using the broad diagnostic threshold (which included BPI, BPII, and UP individuals), and combining the information for the 12 markers, linkage to the whole of chromosome 16 was excluded for models 1 (60% penetrance) and 2 (90% penetrance) assuming a dominant mode of inheritance, and for model 2 assuming a recessive mode of inheritance. The only region not excluded using model 1 with recessive transmission was between D16S402–D16S408, with a maximum lod score of −1.42.

Maximal exclusion of linkage between chromosome 16 and BAD was obtained using the broad disease definition. While exclusion was not always as complete for more stringent diagnostic definitions (BPI and BPII, or BPI only), all lod scores were negative for small values of $\theta$ for all models. Less negative lod scores for the two narrower diagnostic thresholds were not unexpected, given that the number of affected individuals used in the analysis was reduced from 59 for the BPI, BPII, and UP analysis, to 40 for the BPI and BPII analysis, to 35 for the BPI-only analysis. The effect of this reduction was a loss of power for the pedigrees.

Results from APM analysis supported the linkage results, as no significant APM statistics were obtained with the intermediate weighting function $1/\sqrt{p}$. The significant scores observed for the 1/p weighting function were likely to be spurious, as the other two test statistics did not support these findings. APM analyses are extremely dependent on marker allele frequencies and, in particular, the 1/p test statistic heavily weights the allele frequencies [Weeks and Lange, 1988].

The study of Eiberg et al. [1993] suggested evidence for linkage of BAD to PGP (which is located at the same position as D16S291), assuming an autosomal-dominant mode of inheritance. Ewald et al. [1995] reported linkage of BAD to D16S510 (localized to the same region as D16S423) assuming an autosomal-recessive mode of inheritance. In contrast to both of these reports, results obtained in our study excluded linkage of BAD to D16S291 under the assumption of autosomal-dominant inheritance, and to D16S423 with recessive inheritance. Furthermore, linkage to D16S423 and D16S291 was excluded for all thresholds and models. If a susceptibility locus does exist on chromosome 16p13, as suggested by Eiberg et al. [1993] and Ewald et al. [1995], this locus is not responsible for BAD in our Australian families. Our findings are consistent with those of a number of other groups which also examined chromosome 16 markers and which, while not specifically excluding linkage, did not report positive results [Pakstis et al., 1991; Coon et al., 1993; Blackwood et al., 1996; Freimer et al., 1996; Ginns et al., 1996]. This suggests that if this region defines a susceptibility locus, it is a causative locus in only a minority of BAD families.

In a recent critique of molecular genetic studies of BAD, Risch and Botstein [1996] made a number of suggestions pertinent to such ongoing genome screens. One of these was a recommendation that, in addition to the identification of possible susceptibility loci, exclusion maps of the genome need to be constructed. It is critical that findings of nonlinkage, such as our report of chromosome 16, be reported in parallel with positive findings identifying possible new loci.

## ACKNOWLEDGMENTS

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